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## Energy Coupling of Facilitated Transport of Inorganic Ions in *Rhodopseudomonas sphaeroides*

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Within the scope of a study on the effects of changes in medium composition on the proton motive force in *Rhodopseudomonas sphaeroides*, the energy coupling of sodium, phosphate, and potassium (rubidium) transport was investigated. Sodium was transported via an electroneutral exchange system against protons. The system functioned optimally at pH 8 and was inactive below pH 7. The driving force for the phosphate transport varied with the external pH. At pH 8,  $P_i$  transport was dependent exclusively on  $\Delta\psi$  (transmembrane electrical potential), whereas at pH 6 only the  $\Delta pH$  (transmembrane pH gradient) component of the proton motive force was a driving force. Potassium (rubidium) transport was facilitated by a transport system which catalyzed the electrogenic transfer of potassium (rubidium) ions. However, in several aspects the properties of this transport system were different from those of a simple electrogenic potassium ionophore such as valinomycin: (i) accumulated potassium leaked very slowly out of cells in the dark; and (ii) the transport system displayed a threshold in the  $\Delta\psi$ , below which potassium (rubidium) transport did not occur.

In another publication (20a), the electrochemical proton gradient ( $\Delta\mu_{H^+}$ ) generated by light-induced cyclic electron transfer was measured in intact cells and chromatophores of *Rhodopseudomonas sphaeroides*. The magnitude and composition of the ( $\Delta\mu_{H^+}$ ) was strongly dependent on the ionic composition of the medium in which the cells or the chromatophores were suspended. Direct measurements of the effects of different cations and anions on the components of  $\Delta\mu_{H^+}$  have supplied information about the relative permeability of the different ions. In general, cations are less permeant than anions,  $K^+$  is more permeable than  $Na^+$ , and the relative permeability of the anions increases in the following sequence:



Although in these studies no specific attempts were made to unravel the translocation mechanism of these ions, the results indicated that the anions  $SO_4^{2-}$ ,  $Cl^-$ ,  $NO_3^-$ , and  $ClO_4^-$  cross the membrane by passive diffusion, whereas the translocation of  $Na^+$ ,  $K^+$ , and  $HPO_4^{2-}$  is carrier mediated. In this investigation, the mechanism of translocation of  $Na^+$ ,  $K^+$ , and  $HPO_4^{2-}$  and

the role of  $\Delta\mu_{H^+}$  or its components in these translocation processes were studied.

We conclude that  $Na^+$  is translocated by an electroneutral  $Na^+/H^+$  antiport system,  $K^+$  is translocated by electrogenic uniport, and  $HPO_4^{2-}$  is transported by a cation/ $HPO_4^{2-}$  symport system which is electroneutral at pH 6 and electrogenic at pH 8. The translocation systems for these ions in *R. sphaeroides* differ in a number of aspects from analogous systems described for other bacteria.

(The results described in this paper were presented in part at the Third International Congress on Photosynthetic Bacteria in Oxford, U.K. [K. J. Hellingwerf, M. G. L. Elferink, P. A. M. Michels, and W. N. Konings, Abstr. 3rd Int. Symp. Photosynthetic Prokaryotes, abstr. B56, 1979].)

### MATERIALS AND METHODS

**Abbreviations.** Abbreviations used herein are as follows:  $\Delta\mu_{H^+}$ , electrochemical potential gradient for protons or proton motive force;  $\Delta pH$ , transmembrane pH gradient;  $\Delta\psi$ , transmembrane electrical potential;  $\Delta\mu_X$ , chemical potential (concentration) gradient of ion X (in which X can be  $K^+$ ,  $Na^+$ , or  $P_i$ ), SF-6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; MES, 2-N-morpholinoethanesulfonic acid.

**Cell growth.** *R. sphaeroides* strain 2.4.1. was grown anaerobically in the light in the medium of Sistrom at

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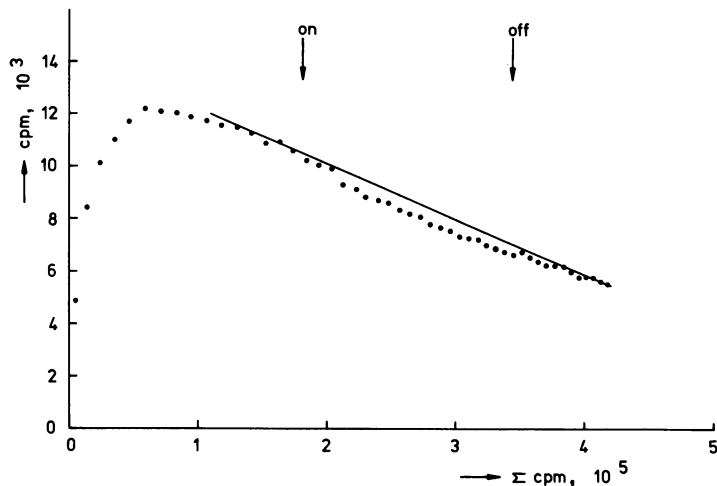


FIG. 1. Light-induced uptake of sodium ions by chromatophores of *R. sphaeroides* as determined by flow dialysis. Chromatophores were prepared in 40 mM potassium glycyglycine (pH 8), 10 mM  $\text{MgSO}_4$ , and 50 mM KCl and suspended in the same medium, supplemented with 1 mM potassium succinate, at a protein concentration of 42.5 mg/ml.  $^{22}\text{NaCl}$  was added to a final concentration of 40  $\mu\text{M}$ . Actinic light was supplied, at saturating intensities, as indicated. Immediately after the light was turned off, carbonylcyanide *m*-chlorophenylhydrazone was added to a final concentration of 100  $\mu\text{M}$ .

30°C as described previously (22). Before harvesting, chloramphenicol (50  $\mu\text{g}/\text{ml}$ ) was added to the cell suspension.

**Isolation of chromatophores.** Chromatophores were isolated from cells, which were washed twice as described (22) and suspended in the media described in Results.

**EDTA treatment of intact cells.** Cells were washed twice, at room temperature, with 100 mM Tris-chloride, pH 8, containing chloramphenicol (50  $\mu\text{g}/\text{ml}$ ) and resuspended in this buffer (1 g [wet weight] per 100 ml). After a 3-min preincubation of the cell suspension at 37°C, sodium EDTA, pH 7, was added to a final concentration of 1 mM. After 2 min, the  $\text{Mg}^{2+}$  concentration was brought to 10 mM. Control cells were obtained by incubating the cells at 37°C for 5 min without EDTA before  $\text{MgSO}_4$  was added.

**Transport studies.** For phosphate transport, EDTA-treated and control cells were washed twice at room temperature with glycyglycine-PIPES buffer at pH 6 or 8 (25 mM glycyglycine plus 25 mM PIPES plus 10 mM  $\text{MgSO}_4$ ;  $\text{K}_2\text{SO}_4$  and KOH were added to obtain the desired pH and a final  $\text{K}^+$  concentration of 100 mM) and finally resuspended in this buffer at a concentration of 0.2 to 0.3 g (wet weight)/ml.

Portions of 0.5 ml were transferred to 25-ml Erlenmeyer flasks. The ionophores valinomycin and nigericin were added as indicated to the final concentrations given in Results. The reaction mixture was kept anaerobic by flushing with oxygen-free nitrogen (13). After 5 min of incubation in the dark, the reaction mixture was illuminated with white light of saturating intensity (approximately 10,000 lx). Subsequently,  $^{32}\text{P}$  (100 Ci/mol) was added to a final concentration of 50  $\mu\text{M}$ . At the times indicated, samples of 100  $\mu\text{l}$  were diluted with 2 ml of 0.1 M LiCl, and uptake of  $^{32}\text{P}$  was measured as described previously (9, 10).

Uptake of  $^{22}\text{Na}$  and  $^{86}\text{Rb}^+$  was measured with the

automated flow dialysis technique described previously (13).

**Protein.** Protein was determined according to Lowry et al. (20).

**Materials.**  $^{32}\text{P}_i$  (carrier-free),  $^{22}\text{NaCl}$  (3,200 Ci/mol), and  $^{86}\text{RbCl}$  (12 Ci/g) were obtained from the Radiochemical Centre (Amersham, Buckinghamshire, England). All other materials were reagent grade and obtained from commercial sources.

## RESULTS

**Sodium transport.** The existence of a sodium ion transport system in *R. sphaeroides* was indicated by the effect of sodium ions on the magnitude and composition of the proton motive force in this bacterium (20a, 24). These observations indicated that sodium ion transport occurs in exchange with protons in a manner similar to that demonstrated in other bacteria (18, 36). The material of choice to study this sodium ion translocation is a preparation of chromatophores, inside-out membrane vesicles (7) which can be prepared from *R. sphaeroides* by French Press treatment (22). In these chromatophores, sodium ions accumulate in response to a proton motive force, acid and positive inside, which can be generated by light-induced cyclic electron transfer (21). Figure 1 shows the results of an experiment in which sodium ion accumulation is followed with automated flow dialyses (13). A sodium ion accumulation ( $\Delta\bar{\mu}_{\text{Na}^+}$ ) of 30 mV could be calculated from this uptake experiment. This sodium accumulation was strictly light dependent and completely sensitive to low

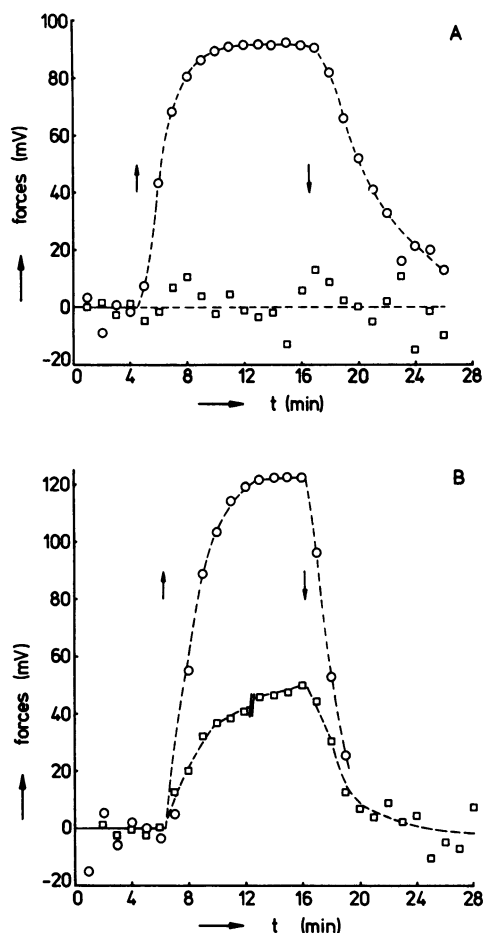


FIG. 2. Effect of ionophores on light-dependent sodium uptake in chromatophores from *R. sphaeroides*. Chromatophores (30 mg of protein/ml) in a buffer containing 100 mM KCl, 1 mM KCN, 1 mM potassium succinate, and 10 mM HEPES, pH 8, were subjected to automated flow dialysis (13). Light of saturating intensity was switched on and off as indicated. For further details on the measurement of the components of the proton motive force, see reference 21. (A) Nigericin (10  $\mu$ M) was present throughout the experiment.  $^{22}\text{NaCl}$  was used at a concentration of 200  $\mu$ M. To dissipate the transmembrane gradients, valinomycin (20  $\mu$ M) and monensin (100  $\mu$ M) were added at the moment the light was switched off. Symbols:  $\square$ ,  $\Delta\mu_{Na^+}$ ;  $\circ$ ,  $\Delta\psi$ . (B) Valinomycin (20  $\mu$ M) was present throughout the experiment. Further conditions were as in (A), except that 10  $\mu$ M nigericin was added instead of 20  $\mu$ M valinomycin. Symbols:  $\square$ ,  $\Delta\mu_{Na^+}$ ;  $\circ$ ,  $\Delta\psi$ .

concentrations of protonophores such as SF-6847 (1  $\mu$ M).

The energy coupling of this sodium ion translocation system and the role of the  $\Delta\text{pH}$  and  $\Delta\psi$  in the uptake of sodium ions were studied (Fig. 2). Valinomycin, in the presence of high concen-

trations of potassium ions, completely dissipates the  $\Delta\psi$ , and consequently the  $\Delta\mu_{H^+}$  across the membrane of the chromatophores consists of a  $\Delta\text{pH}$  only (21). At an external pH of 8, this  $\Delta\text{pH}$  has a magnitude comparable to that of the  $\Delta\mu_{H^+}$  recorded in the absence of ionophore (about +120 mV). This means that in the presence of valinomycin, an appreciable interconversion of  $\Delta\psi$  into  $\Delta\text{pH}$  occurs. The addition of valinomycin stimulated the uptake of sodium ions significantly, and an accumulation as high as 55 mV was obtained (Fig. 2B). This result suggested that the  $\Delta\text{pH}$  and not the  $\Delta\psi$  energized sodium ion uptake and that sodium ion translocation occurred by an electroneutral sodium ion/proton exchange system. This suggestion was further confirmed by the results presented in Fig. 2. In the presence of nigericin, the  $\Delta\text{pH}$  was completely dissipated, and the proton motive force was composed solely of a  $\Delta\psi$ . The interconversion of  $\Delta\text{pH}$  into  $\Delta\psi$  was in this case somewhat less efficient and led to a  $\Delta\psi$  (and thus a  $\Delta\mu_{H^+}$ ) of +95 mV. However, this driving force did not give rise to any uptake of sodium ions (Fig. 2A).

In Table 1, the evidence for an exclusive role of  $\Delta\text{pH}$  as the driving force for  $\text{Na}^+$  uptake is further extended. In these experiments, the relative magnitudes of  $\Delta\text{pH}$  and  $\Delta\psi$  were varied by changing the composition of the medium in which the experiments were performed (20a). The first line of Table 1 shows that a large  $\Delta\psi$  plus a smaller  $\Delta\text{pH}$  led to a low level of sodium ion uptake. When the  $\Delta\text{pH}$  was increased by increasing the anion permeability (from sulfate via chloride to chlorate), a concomitant increase in sodium ion accumulation was observed. Again, dissipation of the  $\Delta\text{pH}$ , by the addition of the weak base ammonium chloride, completely abolished sodium ion uptake.

The same characteristics of sodium ion trans-

TABLE 1. Effect of changes in the composition of  $\Delta\mu_{H^+}$  on the light-driven sodium uptake in chromatophores<sup>a</sup>

Addition	$\Delta\psi$	$\Delta\text{pH}$	$\Delta\mu_{H^+}$	$\Delta\mu_{Na^+}$
Choline sulfate	102	36	138	25
Choline chloride	ND	54		46
Choline chlorate	ND	71		56
Ammonium chloride	133	10	133	5

<sup>a</sup> Chromatophores were prepared and assayed for transmembrane gradients, with the use of automated flow dialysis (13), in a buffer containing 25 mM choline sulfate, 2 mM choline succinate, and 50 mM HEPES, pH 8.0. The additions were made to the upper compartment of the flow dialysis vessel to final concentrations of 50 mM. All other conditions were as described in the legend to Fig. 2. Values are given in megavolts; ND, not determined.

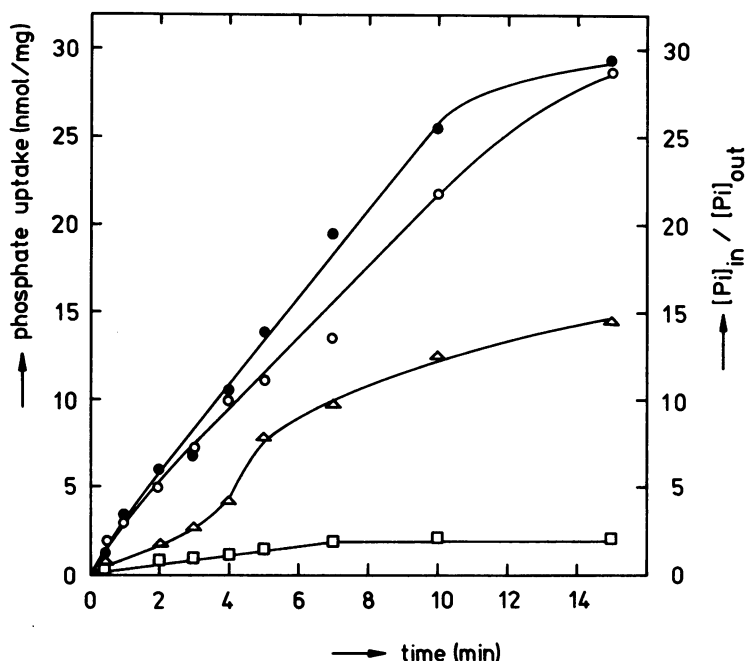


FIG. 3. Active transport of  $P_i$  into *R. sphaeroides*. A cell suspension (0.79 mg of protein/ml) in 50 mM potassium glycyglycine buffer, pH 8, plus 10 mM  $MgSO_4$  was incubated under nitrogen at 30°C at saturating light intensity for 5 min. Phosphate uptake was initiated through addition of  $^{32}P_i$  (100 Ci/mol; final concentration, 50  $\mu M$ ). Samples of 200  $\mu l$  were withdrawn at the indicated times, diluted 1:10 with 0.1 M LiCl, filtered immediately, and washed with 2 ml of 0.1 M LiCl. In a parallel experiment, the filters were extracted with 1 ml of 5% TCA for 20 to 30 min at 4°C. The extract was fractionated into organic phosphate and orthophosphate according to Avron (1). Radioactivity was measured by scintillation spectrometry. The internal concentration of orthophosphate was calculated by using an internal *R. sphaeroides* volume of 2.3  $\mu l$  per mg of protein (20a). Symbols:  $\circ$ , total phosphate;  $\Delta$ , TCA-soluble organic phosphates;  $\square$ , orthophosphate;  $\bullet$ , ratio  $[P_i]_{in}/[P_i]_{out}$ .

location in chromatophores of *R. sphaeroides* were observed at pH 7. At lower pH values, however, no significant translocations of sodium ions could be detected.

**Phosphate transport.** The mechanism of  $P_i$  transport was studied in intact cells of *R. sphaeroides* and not in membrane vesicles, since membrane vesicles with high transport activities can be prepared only in phosphate buffer. Uptake of  $P_i$  by intact cells was observed under a wide range of conditions. For instance,  $P_i$  uptake by *R. sphaeroides* was seen in media with pH values between 6 and 8 which contained one or more different anions and cations and after pretreatment of the cells with EDTA. To demonstrate active uptake of  $P_i$ , it was necessary to trace the fate of the internalized phosphate. The total amount of phosphate, which entered *R. sphaeroides* under energized conditions, increased approximately linearly with time for about 10 min, representing a rate of phosphate transport of 2.5 nmol/min per mg of protein (Fig. 3). Most of this  $P_i$  was incorporated rapidly into cold trichloroacetic acid (TCA)-soluble organic phosphate(s) and into TCA-insoluble phos-

phates. A minor fraction was present in the cells as  $P_i$ . With the known internal volume of these cells (2.3  $\mu l$ /mg protein [20a]), the maximal concentration gradient during energization was  $[P_i]_{in}/[P_i]_{out} = 29$ , representing a  $\Delta\bar{\mu}_{P_i}$  of 88 mV.

We studied the energy coupling of phosphate

TABLE 2. Effect of ionophores on  $P_i$  uptake in *R. sphaeroides*<sup>a</sup>

Addition	Concn ( $\mu M$ )	Initial rate of $P_i$ uptake (nmol/min per mg)	
		pH 8	pH 6
None		1.30 (100) <sup>b</sup>	0.43 (100)
Valinomycin	1	0.05 (4)	1.00 (233)
	3	0.03 (2)	0.67 (156)
Nigericin	0.2	1.66 (128)	0.23 (53)
	0.6	1.60 (123)	0.12 (28)

<sup>a</sup> The initial rate of uptake (1-min measurements) of  $P_i$  was measured as described in the legend to Fig. 3. Preincubation with ionophores: 5 min in the dark at 30°C.

<sup>b</sup> Numbers in parentheses indicate percentage of the rate of uptake in the absence of ionophores.

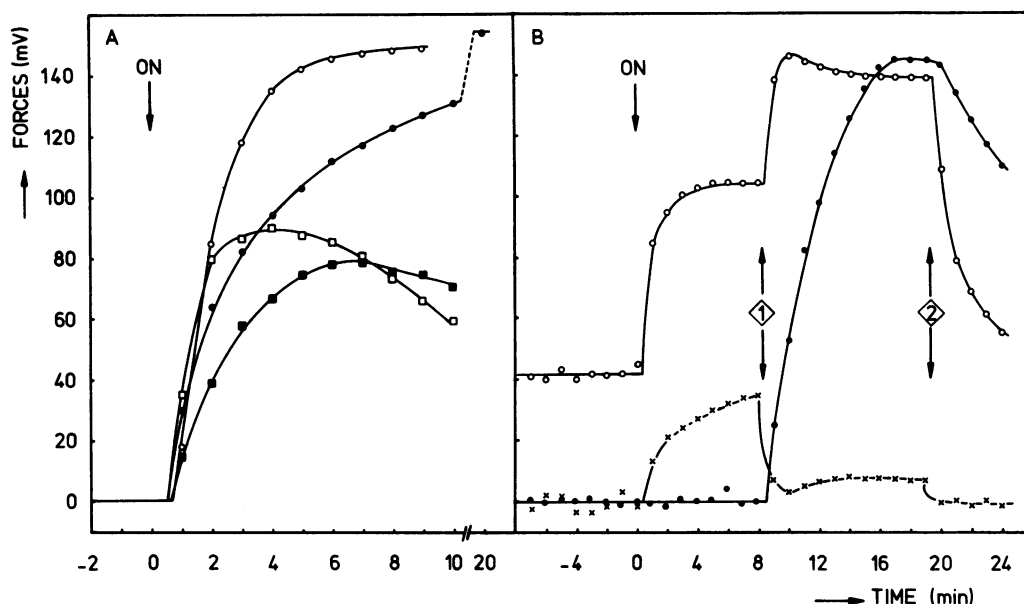


FIG. 4. Comparison of  $\Delta\mu_{\text{Rb}^+}$  and  $\Delta\psi$ , measured with  $^{86}\text{Rb}^+$  plus valinomycin.  $\text{Rb}^+$  accumulation in intact cells of *R. sphaeroides* was measured with automated flow dialysis (13) in the presence and absence of 20  $\mu\text{M}$  valinomycin (preincubation for at least 1 h at 25°C) to measure  $\Delta\psi$  and  $\Delta\mu_{\text{Rb}^+}$ , respectively. Light of saturating intensity was switched on as indicated. Before the measurements, the cells were washed extensively (five to eight times in a period of 2 days and at room temperature) to deplete the endogenous energy reservoirs (23). (A) A medium containing 67 mM  $\text{Na}_2\text{SO}_4$ , 20 mM MES, 20 mM HEPES, 5 mM  $\text{MgSO}_4$ , 2 mM sodium EDTA, and 15  $\mu\text{g}$  of chloramphenicol per ml was used. Open symbols, plus valinomycin; closed symbols, without valinomycin; squares, pH 5.5; circles, pH 7.5. (B) A medium containing 100 mM choline chloride, 20 mM PIPES, 5 mM  $\text{MgSO}_4$ , and 10  $\mu\text{g}$  of chloramphenicol per ml, adjusted to pH 7.5 with choline base, was used. Symbols:  $\circ$ ,  $\Delta\psi$ ;  $\bullet$ ,  $\Delta\mu_{\text{Rb}^+}$ ;  $\times$ ,  $\Delta\text{pH}$ . At arrow 1, 35 mM sodium formate, pH 7.5 was added to the upper compartment of the flow dialysis vessel. At arrow 2, the light was switched off.

transport in an experiment in which the effect of ionophores on the initial rate of  $\text{P}_i$  uptake was measured in EDTA-treated *R. sphaeroides* at pH 8 and pH 6 (Table 2). The EDTA treatment was given to facilitate incorporation of the ionophores into the cytoplasmic membrane of this bacterium. At pH 8, uptake of  $\text{P}_i$  was completely inhibited by valinomycin and slightly stimulated by nigericin. At pH 6, the situation was more complex. The overall rate of  $\text{P}_i$  uptake at this pH was about threefold lower than at pH 8. Furthermore, phosphate uptake was stimulated (about twofold) by valinomycin and inhibited by nigericin. An inhibition of 80% was exerted by 1  $\mu\text{M}$  nigericin. Under all of these experimental conditions, the  $\text{P}_i$  uptake was completely sensitive to moderate concentrations of the protonophore SF-6847 ( $\pm 10 \mu\text{M}$ ).

**Potassium (rubidium) ion transport.** Potassium ion transport in *R. sphaeroides* has been studied through measurement of  $\text{Rb}^+$  uptake (16) (see below and Discussion). Active uptake of  $\text{Rb}^+$  into *R. sphaeroides* can be observed in all stages of growth. However, the rate of  $\text{Rb}^+$  uptake varies significantly during growth. Maximal  $\text{Rb}^+$

uptake rates (approximately 6 nmol/min per mg of protein) are obtained with cells harvested at the late exponential phase of growth.

$\text{Rb}^+$  uptake could be observed in a broad pH range (Fig. 4A). The  $\text{Rb}^+$  uptake rate in cells suspended in 100 mM choline chloride plus 20 mM PIPES decreased gradually with the external pH. At pH 5.5, the rate was about 25% of the rate at pH 7.5.

The  $\text{K}^+$  ( $\text{Rb}^+$ ) uptake system was very specific for  $\text{Rb}^+$  and potassium ions. The initial rate of  $\text{Rb}^+$  uptake, as measured by the accumulation of  $^{86}\text{Rb}^+$  with automated flow dialysis (13), was inhibited completely by 3.2 mM potassium chloride or rubidium chloride (approximately a 20-fold excess of the unlabeled cations), 75% by 3.2 mM  $\text{Ti}^+$ , and very little (less than 25%) by 3.2 mM  $\text{Li}^+$  or  $\text{Cs}^+$ .

The functioning of the potassium ion transport system in *R. sphaeroides* appeared to be dependent on an intact outer membrane. EDTA treatment (10 mM EDTA for 30 min at 30°C; 35) completely abolished rubidium uptake, and this uptake could not be restored by several washings of the bacterial suspension with media

containing excess magnesium ions. In contrast, treatment of the bacteria with low concentrations of EDTA (up to 2 mM at 20°C for 30 min) did result in a reversible inhibition of  $\text{Rb}^+$  uptake (see below).

The energy coupling of  $\text{K}^+$  uptake was studied in intact cells in a series of experiments with inhibitors and ionophores. First, we studied the effect of arsenate to investigate whether "phosphate bond" energy contributed to the energy used to accumulate  $\text{Rb}^+$ . Overnight incubation of a cell suspension with 5 mM sodium arsenate (2-h illumination followed by storage at room temperature in the dark) in a medium with 100 mM choline chloride, 20 mM PIPES, and 5 mM  $\text{MgSO}_4$ , pH 7.5, did not affect  $\text{Rb}^+$  uptake in the light in any observable way. In addition, an unimpaired light-dependent  $^{86}\text{Rb}^+$  uptake was observed after extensive treatment of the cells with dicyclohexylcarbodiimide (50  $\mu\text{M}$  for 2 h at 30°C, in the presence of 1 mM EDTA). This treatment completely blocked the intracellular ATP synthesis via the membrane-bound ATPase complex (data not shown). On the other hand, addition of protonophorous uncouplers severely inhibited rubidium ion uptake: 50% inhibition was obtained with 10  $\mu\text{M}$  SF-6847.

These results suggested that  $\text{Rb}^+$  was taken up by *R. sphaeroides* in response to a light-induced  $\Delta\bar{\mu}_{\text{H}^+}$ , without the involvement of phosphate bond energy (see Discussion). A study of the role of the components of the  $\Delta\bar{\mu}_{\text{H}^+}$  as a driving force for  $\text{K}^+$  transport was complicated by the fact that the ionophores nigericin and valinomycin facilitated transmembrane transfer of  $\text{Rb}^+$  and  $\text{K}^+$ . With high concentrations of valinomycin, the transmembrane electrical potential could be calculated from the distribution of  $^{86}\text{Rb}^+$ .  $\text{Rb}^+$  accumulation in the presence and absence of valinomycin (to measure  $\Delta\psi$  and  $\Delta\bar{\mu}_{\text{Rb}^+}$ , respectively) was studied in untreated cells, in EDTA-pretreated cells, and in cells to which EDTA was added at the start of the experiments. Furthermore, media of different ionic composition and pH were used. In general, the steady-state levels of  $\text{Rb}^+$  accumulation in the presence or absence of the ionophore were equal (Fig. 4A), indicating that only the  $\Delta\psi$  was the driving force for  $\text{Rb}^+$  uptake. The rate of  $\text{Rb}^+$  uptake, however, was strongly stimulated by valinomycin (about fourfold in Fig. 4A). Also,  $\text{Rb}^+$  efflux was strongly stimulated by valinomycin. Under some experimental conditions, however, large differences were observed between the steady-state levels of  $\text{Rb}^+$  accumulation in the presence and absence of valinomycin, indicating that in the absence of valinomycin  $\text{Rb}^+$  did not always equilibrate with the  $\Delta\psi$  (Fig. 4B). In cells washed and suspended in 100 mM choline chloride, pH 7.5, no accumulation

of  $\text{Rb}^+$ , in the absence of valinomycin, was observed. Rubidium uptake could be restored by adding 35 mM sodium formate, and  $\Delta\bar{\mu}_{\text{Rb}^+}$  equilibrated in the absence of valinomycin with  $\Delta\psi$ . These results could not be explained by the presence of a second, sodium-dependent  $\text{Rb}^+$  uptake system such as the  $\text{Na}^+$ ,  $\text{K}^+$  ( $\text{Rb}^+$ )/ $\text{H}^+$  antiport system found in *Escherichia coli* (cf. reference 34) because all experiments to demonstrate such a system failed: (i)  $\text{Rb}^+$  uptake at the different stages of growth did not depend on the presence of  $\text{Na}^+$ ; (ii) proton movements could not be detected after applying pulses of potassium to an unbuffered cell suspension, also in the absence of  $\text{Na}^+$ ; (iii) sodium-dependent rubidium uptake in exchange for protons was not observed in chromatophores in which a  $\Delta\bar{\mu}_{\text{H}^+}$ , composed of mainly of a  $\Delta\text{pH}$ , was present. Such conditions were attained by illuminating chromatophores suspended in 100 mM choline chlorate (20a). These results are more in line with an electrogenic  $\text{Rb}^+$  transport system in these membranes than with a system in which proton antiport is involved.

This idea was confirmed by the observation that  $\text{Rb}^+$  uptake occurred at a high rate when the  $\Delta\text{pH}$  was completely converted to  $\Delta\psi$ . This could be achieved by adding sodium formate to the incubation mixture (Fig. 4B). Another possible explanation for the results of Fig. 4B is that potassium ion transport occurred via an electrogenic transport system the rate of which is an alinear function of its driving force. The results of Fig. 5 support this explanation. The magnitude of the  $\Delta\psi$  was varied by changing the intensity of the actinic light. Below  $-120$  mV almost no uptake of  $\text{Rb}^+$  occurred, whereas a large increase in the uptake rate was observed when the  $\Delta\psi$  increased to  $-180$  mV.

## DISCUSSION

In this study, we investigated the energy coupling of transport of inorganic ions across the *R. sphaeroides* membrane for those ions which are quantitatively most important, i.e.,  $\text{Na}^+$ , phosphate, and  $\text{K}^+$  ( $\text{Rb}^+$ ). In addition to these systems, transport systems for divalent ions have been reported.  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$  are usually accumulated in bacterial cells, whereas  $\text{Ca}^{2+}$  is extruded (17), as has also been demonstrated in *R. sphaeroides* (22). In most bacteria,  $\text{Ca}^{2+}$  extrusion occurs in antiport with protons (33). For anions, facilitated transport systems might exist (e.g., for sulfate or nitrate), but no evidence for *R. sphaeroides* is available on this point.

The results indicate that sodium ions are transported by a sodium/proton antiport system which catalyzes the facilitated electroneutral exchange of protons against sodium ions. If

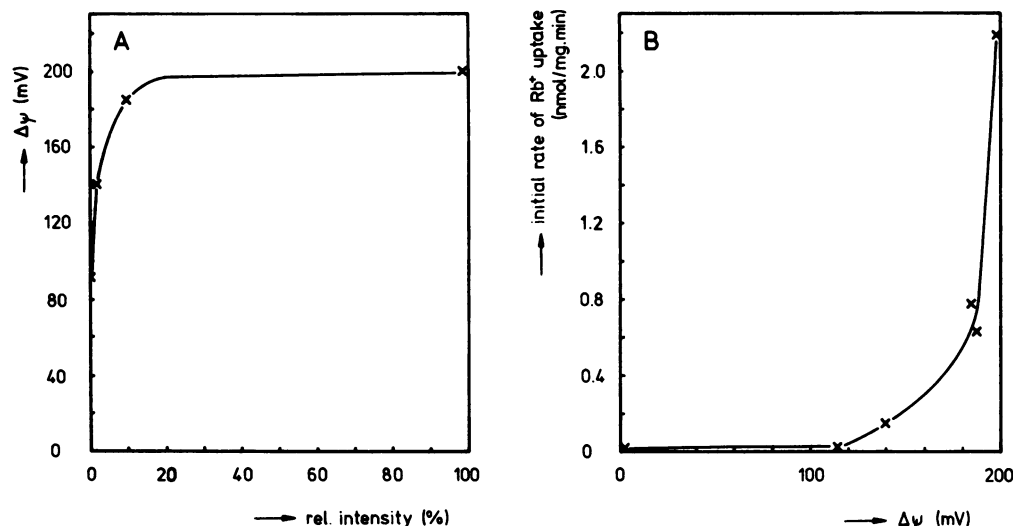


FIG. 5. Relationship between the rate of  $^{86}\text{Rb}^+$  uptake and its driving force. A medium containing 67 mM  $\text{Na}_2\text{SO}_4$ , 40 mM sodium phosphate, 1 mM  $\text{MgSO}_4$ , and 15  $\mu\text{g}$  of chloramphenicol per ml, adjusted to pH 8 with  $\text{NaOH}$ , was used. The cells were washed seven times in a period of 2 days, at room temperature, before the measurements. The driving force for the  $^{86}\text{Rb}^+$  uptake was varied by changing the intensity of actinic illumination. Both  $\Delta\psi$  and the rate of  $\text{Rb}^+$  uptake were assayed with the use of automated flow dialysis (13).

more than one proton were exchanged per sodium ion, uptake of sodium ions in chromatophores would be driven by an electrical potential only. This was not observed (Fig. 2A and Table 1). In the inverse situation (more than one sodium ion per proton), uptake of sodium ions would not occur in chromatophores, in which the  $\Delta\psi$  exceeds the  $\Delta\text{pH}$ . The activity of the  $\text{Na}^+/\text{H}^+$  exchange system is not linked to energy derived from high-energy phosphate bonds (4, 14, 15), as these compounds are not present in the chromatophore preparations. A satisfactory explanation for the large difference between  $\Delta\text{pH}$  and  $\Delta\bar{\mu}_{\text{Na}^+}$  cannot be given. To some extent, this is due to the fact that in our experiments a steady-state level of  $\text{Na}^+$  accumulation was not established. Also, nonfacilitated electrogenic leakage of sodium ions could lead to a lower steady-state level of accumulation.

The existence of an electroneutral sodium/proton antiport system is also indicated by the results presented in Fig. 2. These results cannot be explained by a valinomycin- or nigericin-mediated sodium translocation across the chromatophore membrane. The same results were obtained when no ionophores were used (Table 1). Furthermore, a facilitated transport of  $\text{Na}^+$  via the ionophores would give effects opposite those observed in Fig. 2. The presence of a  $\text{Na}^+/\text{H}^+$  antiport system in *R. sphaeroides* was already deduced from observations on the effects of different media on the  $\Delta\text{pH}$  in this organism (24). Also, these observations indicated a de-

creased activity of this system at lower pH values.

The specificity of the proton/sodium exchange system for different cations could, indirectly, be inferred from the effect of the cations on  $\Delta\text{pH}$ ,  $\Delta\psi$ , or rubidium uptake in intact *R. sphaeroides* cells (see also below). This system appeared to be very specific for  $\text{Na}^+$ . The cations  $\text{Cs}^+$ ,  $\text{Li}^+$ , choline $^+$ , and  $\text{Tris}^+$  were clearly not transported by this exchange system. Transport of  $\text{K}^+$ ,  $\text{Rb}^+$ , and  $\text{Tl}^+$  by this exchange system could not be excluded due to the presence of an efficient potassium transport system. However, all attempts to demonstrate the existence of another cation/proton exchange system by 9-amino-acridine fluorescence measurements in a way similar to that described for inverted membrane vesicles isolated from *E. coli* (5) were unsuccessful (K. J. Hellingwerf and W. N. Konings, unpublished data).

In two other bacteria, the  $\text{Na}^+/\text{H}^+$  exchange system has been studied extensively. Both systems behave differently from the system in *R. sphaeroides*. In *E. coli*, a system is present which catalyzes an electroneutral exchange at low external pH values and an electrogenic exchange (the number of protons exceeds the number of sodium ions) at higher external pH (>6.5). In *Halobacterium halobium*, an exchange system has been reported which catalyzes an electrogenic exchange of  $\text{H}^+$  against  $\text{Na}^+$  under all conditions tested (6, 18).

The transport system for phosphate in *R.*



*sphaeroides* is the only system studied in this investigation which shows a characteristic external pH dependence in its mode of energy coupling. At low pH, phosphate transport is mainly  $\Delta\text{pH}$  driven, which suggests that  $\text{H}_3\text{PO}_4$  is the transported species. This is in agreement with observations on phosphate transport in *Micrococcus lysodeikticus* (9, 10) and with observations of Russel and Rosenberg on  $\text{P}_i$  transport in *E. coli* (31) by the PIT transport system. Also, in *Streptococcus faecalis* phosphate transport has been reported to occur effectively as  $\text{H}^+/\text{H}_2\text{PO}_4^-$  symport (11). In *R. sphaeroides* at pH 8, phosphate transport must occur in the  $\text{HPO}_4^{2-}$  form in symport with cations, which cannot be protons and which give the overall translocated complex an excess positive charge. The nature of these cations still remains to be established. This conclusion is, of course, valid only if no more than one transport system for  $\text{P}_i$  is operative in this bacterium.

$\text{P}_i$  transport in *R. sphaeroides* is not driven by high-energy phosphate bond energy. The addition of only valinomycin or nigericin causes an interconversion of the components of the proton motive force with little or no decrease of the total  $\Delta\mu_{\text{H}^+}$  (12, 26). The effects of these interconversions on high-energy phosphate bonds would be small, if not absent. However, both ionophores affect strongly the uptake of phosphate.

Information on the mechanism of potassium transport was obtained by using rubidium as the potassium analog. Differences between transport of potassium and rubidium (28) have been reported. However, Jasper (16) has shown convincingly that rubidium ions can be taken as an indicator for potassium ion transport in *R. capsulata*. We observed that the inhibitions of  $^{86}\text{Rb}^+$  uptake by unlabeled potassium and rubidium were indistinguishable at a 3.2 mM concentration of these cations.

In  $\text{Rb}^+$  uptake in *R. sphaeroides*, phosphate bond energy is not involved: we observed no effect of pretreatment with arsenate, a treatment which is frequently used to demonstrate the involvement of phosphate bond energy (30), or with dicyclohexylcarbodiimide; also, rubidium ion uptake is sensitive to low concentrations of protonophores. In *E. coli*, four different potassium transport systems have been observed (27). In the quantitatively most important TrkA and Kdp systems, phosphate bond energy has been implied in the mechanism of transport (2, 28). The TrkA system catalyzes a symport reaction between  $\text{K}^+$  and a proton (2). In *E. coli* mutants which contain only the TrkA potassium transport system,  $\Delta\mu_{\text{Rb}^+}$  can therefore be significantly higher than  $\Delta\psi$  (2). Also, in *S. faecalis* potassium transport appears to be driven by phosphate bond energy (30). In *H. halobium*, an

electrogenic potassium transport system (25) similar to the *R. sphaeroides* system has been described. Sorensen and Rosen recently reported (34) the sodium dependence of rubidium (potassium) ion transport in *E. coli*. Our observations (Fig. 4B) strongly indicate that conclusions about specific involvement of sodium ions in the transport of rubidium can be drawn only after the effect of sodium ions on the composition of the proton motive force has been established.

The absolute threshold value in  $\Delta\psi$  for rubidium transport in intact *R. sphaeroides* cells cannot yet be given. The value given in Results is based on the assumption that none of the  $\text{Rb}^+$  ions accumulated inside the cells are bound to cellular constituents. This assumption, which frequently is made in bioenergetic studies, is subject to some criticism. Recently, we found that this assumption is certainly not correct for another indicator of the membrane potential, tetraphenylphosphonium (J. S. Lolkema, B. ten Brink, K. J. Hellingwerf, and W. N. Konings, unpublished data). These problems do not invalidate the conclusion of equilibrium between  $\Delta\mu_{\text{Rb}^+}$  and  $\Delta\psi$ , since the same corrections for both parameters will be required. Furthermore, it is important to point out that the threshold  $\Delta\psi$  value for  $\text{Rb}^+$  uptake appears to be pH dependent, since a considerable rate of rubidium ion uptake is already observed at pH 5.5 with an electrical potential of  $-90$  mV, whereas at pH 8 a threshold  $\Delta\psi$  value of  $-120$  mV is needed. At this moment, we cannot give a firm explanation for this phenomenon. It is possible that these changes are caused by changes in the intracellular redox potential (cf. reference 29). This problem has now been investigated in more detail.

Threshold values for the uptake of solutes as a function of their driving force have been reported for  $\text{Na}^+$ ,  $\text{H}^+$  exchange, and amino acid transport in *H. halobium* (19) and alanine uptake in membrane vesicles of *R. sphaeroides* (I. Friedberg, K. J. Hellingwerf, and W. N. Konings, Abstr. 13th Fed. Eur. Biochem. Soc. Meet., abstr. S5-P77, 1980). When potassium uptake in *H. halobium* was measured as a function of light intensity, a slightly sigmoidal curve was obtained (25). Usually a hyperbolic relation is obtained between light intensity and proton motive force generation (12, 25), and a threshold in  $\text{Rb}^+$  ( $\text{K}^+$ ) uptake could therefore be the explanation for the sigmoidal  $\text{Rb}^+$  uptake curve in this halophilic bacterium. A molecular interpretation for the threshold in rubidium uptake rate cannot be given at this moment.

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